PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Robert J. Harman et al.
Application No.	:	10/575,063
Filed	:	February 1, 2007
For	:	METHODS OF PREPARING AND USING NOVEL STEM CELL COMPOSITIONS AND KITS COMPRISING THE SAME
Examiner	:	Lora Elizabeth Barnhart
Art Unit	:	1651
Docket No.	:	930120.401USPC

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF JEFFREY CATANIA, PH.D. PURSUANT TO 37 C.F.R. §1.132

I, Jeffrey Catania, declare as follows:

1. I currently hold the position of Scientist, Level 1, at Vet-Stem, Inc. and have been employed by Vet-Stem, Inc. since May 2008. I was awarded a Doctor of Philosophy degree in Pharmacology and Toxicology from The University of Arizona in 2006 and was a post-doctoral fellow at Texas A&M University from 2006 to 2008. Prior to entering graduate school, I was a double-major in Chemistry and Biochemistry at The University of Arizona, receiving a Bachelor of Science Degree in 1997.

2. I am familiar with the content of the above-identified patent application ("the Application"), and I have reviewed the Office Action dated August 28, 2009, including the Examiner's comments regarding the alleged obviousness of certain methods of preparing a purified cell population claimed in the Application. I submit this Declaration for the purpose of providing evidence demonstrating that the presently claimed methods provide surprising and unexpected advantages over the prior art. Specifically, the methods of the present invention, which do not include a step to remove red blood cells, result in a greater yield of nucleated cells

as compared to comparable methods that include a step to remove red blood cells. The experiments described herein were performed by myself or under my supervision.

3. Adipose tissue samples were obtained from five canines and processed as described in the Application. Briefly, samples of canine adipose tissue were obtained from donor dogs under the control of an animal shelter. The samples were collected from each donor and placed in a Vet-Stem tissue collection kit and returned to Vet-Stem via courier. Each sample was removed from the transport tubes, weighed and minced. Digestion was conducted according to protocol with an enzyme cocktail consisting of collagenase and hyaluronidase for a total of 50 minutes at 37 °C. The digested tissue was centrifuged, the lipid and supernatant were removed, and the pellet was resupsended in PBS. Each pellet was processed three times with PBS and centrifugation resulting in a washed, nucleated cell preparation. A total nucleated cell count for each sample was performed using the NucleoCounter, an automated cell counter that does not enumerate red blood cells. The cells from each sample were then split evenly amongst two tubes based upon the nucleated cell count. Once split, the distributed cells are then brought up to a final volume of approximately 2.0 mL in PBS, and an "initial" nucleated cell count for each aliquot was performed. The tubes were then processed in parallel. One of the tubes for each sample remained at room temperature, while the other tube for each sample was subjected to red blood cell lysis. Red blood cell lysis was performed by centrifuging the cells at 1200 × *g* for 10 min. After removal of the supernatant, the pellet was resuspended in 5 mL of 160 mM NH_4Cl (made fresh each day) and allowed to incubate for 10 min. The aliquot was then brought up to 50 mL total with PBS and centrifuged again at 1200 × *g*. The supernatant was removed, and the final cell pellet was resuspended in approximately 2 mL of PBS. At the completion of the red blood cell lysis procedure, a second, "final" (post-treatment) nucleated cell count was performed, and a sample from each aliquot containing 500,000 nucleated cells was placed in a final volume of 1.0 mL PBS and submitted for cytological analysis by STAT Veterinary Laboratory (San Diego, CA), where nucleated cell preparations were analyzed via a Beckman-Coulter hematology analyzer. Data was determined as the average and standard deviations for each procedure, *i.e.*, no treatment with NH_4Cl or treatment with NH_4Cl. Differences between the two

procedures were calculated for each individual adipose sample and subsequently averaged with the other adipose samples.

4. The difference between the initial nucleated cell count and the final nucleated cell count for cells that were not treated with NH_4Cl ranged from 70.84% to 93.21%, whereas for the cells treated with NH_4Cl to lyse red blood cells, the values ranged from 49.47% to 74.14% (Table 1; "VS" indicates samples not treated with NH_4Cl). When the average and standard deviations between the two procedures were calculated, sample aliquots processed without NH_4Cl had 20.87% ± 10.21% more nucleated cells than sample aliquots processed with NH_4Cl treatment. The difference between the two procedures ranged from 7.61% to 35.71% more nucleated cells in samples processed without NH_4Cl treatment, which indicates that a significant portion of the total nucleated cell population is lost in the process of lysing the red blood cells. This also indicates that the lysing of red blood cells adds a level of variability not present in the absence of red blood cell lysis, such that methods that do not involve lysing red blood cells provide a more consistent final nucleated cell yield.

5. As expected, red blood cell lysis greatly decreased the number of red blood cells, as indicated by the average for the VS Procedure of 28,000,000 red blood cells per aliquot versus 12,000,000 red blood cells average for the RBC Lysis Procedure (Table 1).

Table 1.

Procedure	Percentage of Final Cell Count to Initial Cell Count				Per-Sample Difference of Final Cell Count to Initial Cell Count Percentage (VS - RBC Lysis)			
	Average	Std. Dev.	Range		Average	Std. Dev.	Range	
n = 5			Min	Max			Min	Max
VS	82.53	8.10	70.84	93.21	20.87	10.21	7.61	35.73
RBC Lysis	61.66	8.86	49.47	74.14				

Procedure	Red Blood Cell Count (Cells per Cytology Aliquot)				Per-Sample Difference in Red Blood Cell Count (VS - RBC Lysis)			
	Average	Std. Dev.	Range		Average	Std. Dev.	Range	
n = 5			Min	Max			Min	Max
VS	28,000,000	13,038,405	10,000,000	40,000,000	16,000,000	8,944,272	10,000,000	30,000,000
RBC Lysis	12,000,000	10,954,451	0	30,000,000				

6. Accordingly, I submit that the presently claimed methods offer significant unexpected advantages over prior art methods of processing adipose tissue to obtain purified cell populations comprising adipose tissue-derived stem cells, since they yield a significantly greater number of nucleated cells and provide a more consistent nucleated cell preparation. This, of course, is tremendously advantageous, considering the limited tissue source for obtaining such cells, and the desire to administer a large number of stem cells for therapeutic purposes.

I hereby declare that all statements made herein are, to my own knowledge, true and that all statements made on information or belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the captioned patent application or any patent issued therefrom.

Date 01/25/2010

Jeffrey Catania, Ph.D.